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Antiviral Activity of Ammonium Picrate in Mice. (30752)

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In the course of screening compounds for antiviral activity in mice, ammonium picrate was found to be uniquely effective against the Columbia SK virus (encephalomyocarditis) infection. Results of experiments characterizing the antiviral properties of this compound are reported here.

Materials and methods. The efficacy of ammonium picrate was evaluated in Taconic Farms male albino adult mice infected subcutaneously or intracerebrally with aqueous dilutions of SK virus. Stock virus was the supernatant derived by centrifugation from a 10% aqueous suspension of homogenized pooled infected brain tissue. Infectivity of stock virus was such that 90 to 95% of the nontreated mice infected subcutaneously with 0.2 ml of a 10^{-6} dilution died, with a mean survival time of 4.5 days. The mortality of mice infected with a 10^{-7} dilution was approximately 50%. Ammonium picrate was lethal at 200 mg/kg given as a single dose by either intraperitoneal or subcutaneous injection; a dose of 100 mg/kg by these routes was tolerated with no overt ill effects. A single oral dose of 400 mg/kg administered by stomach tube was lethal; a dose of 200 mg/kg by this route was tolerated. Treatment with ammonium picrate by injection or by oral tubing was given at 24 hours and 2 hours before infection, and again at 24 hours after infection, unless otherwise specified. The activity of ammonium picrate in infected mice was measured routinely in terms of the number of animals surviving on the 21st day postinfection.

TABLE I. Effect of Ammonium Picrate on Survival of Mice After Subcutaneous Infection with Columbia SK Virus.

Drug dose* (mg/kg)	Survivors/total mice on 21st day: Intraperitoneal	Subcutaneous	Oral tubing
400	—	—	Toxic
200	Toxic	Toxic	19/19
100	94/100	19/20	19/19
50	66/80	13/20	17/20
25	48/100	5/20	8/20
12	24/80	6/20	4/20
6	—	4/20	7/20
0	6/120	2/20	2/20
ED_{50}, mg/kg:	22 (18–27)	29 (22–39)	26 (20–34)

* Dose given by indicated routes at 24 hr and 2 hr before, and at 24 hr after subcutaneous infection with 0.2 ml of 10^{-6} dilution of stock virus.

Results. Mice infected subcutaneously with a 10^{-6} dilution of stock virus responded to either parenteral or oral treatment with ammonium picrate (Table I). Graded dosage of compound produced a graded effect. Maximum tolerated dose levels protected 95 to 100% of the infected mice. All treated mice surviving the infection remained free of any symptoms of the infection or ill effects of treatment and were, on visual inspection, indistinguishable from the noninfected controls. The median effective dose (ED_{50}) for each route of treatment, based on at least 20 mice per dose level, was calculated by the method of Litchfield and Wilcoxon(1).

The effectiveness of ammonium picrate, at maximum tolerated intraperitoneal dosage, was tested against graded doses of SK virus injected subcutaneously. The results of this experiment show that the larger the infecting

TABLE II. Effect of Ammonium Picrate on Survival of Mice After Subcutaneous Infection with Graded Doses of Columbia SK Virus.

Virus dilution	Survivors/total mice on 21st day	
	Treated*	Nontreated
10^{-4}	5/20	0/20
10^{-5}	14/20	0/40
10^{-6}	19/20	3/40
10^{-7}	20/20	16/40

* 100 mg/kg given intraperitoneally at 24 hr and 2 hr before, and at 24 hr after subcutaneous infection with 0.2 ml of indicated dilutions of stock virus.

dose the less effective the compound (Table II). However, significant protection was obtained over at least a 100-fold range of size of infecting dose.

Examination of the effect of varying the number of intraperitoneal doses of ammonium picrate, and varying the time of starting treatment, revealed that a single dose, if given within a 4-hour period before infection, or at the time of infection, produced the optimum effect. Treatment delayed for 1 hour after infection was not effective.

The efficicay of ammonium picrate was tested in mice infected intracerebrally with 0.03 ml of a $10^{-5.3}$ dilution of stock virus. For comparison, the compound was also tested against an infection produced by subcutaneous injection of 0.2 ml of the same virus dilution. Single dose treatment intraperitoneally at 2 hours before infection was effective against infection by either route (Table III).

To establish whether or not an infection aborted by treatment with ammonium picrate had rendered the surviving mice immune, the survivors of one experiment were reinfected. The survivors were grouped according to the amount of drug used to protect against the initial infection. These groups were then divided in 2 subgroups, one of which was challenged with a 10^{-5} and the other with a 10^{-6} dilution of stock virus. Control mice which had not been subjected to the original infection served as infected controls for the challenge infections. All groups of mice, irrespective of previous treatment, were equally susceptible, as indicated by mortality and mean survival time, and were judged to be nonimmune.

TABLE III. Effect of Ammonium Picrate on Survival of Mice After Subcutaneous or Intracerebral Infection with Columbia SK Virus.

Drug dose* (mg/kg)	Survivors/total mice on 14th day	
	Subcutaneous infection	Intracerebral infection
100	39/40	27/40
50	23/40	20/40
25	11/40	7/40
12	0/30	5/30
0	0/40	2/40
ED_{50}, mg/kg:	43 (38–49)	45 (35–38)

* Single intraperitoneal dose given at 2 hr before subcutaneous infection with 0.2 ml or intracerebral infection with 0.03 ml of $10^{-5.3}$ dilution of stock virus.

The antiviral effect of ammonium picrate, at maximum tolerated intraperitoneal dosage, was examined in 5 additional virus test systems: (1) intranasal influenza type A (PR8) infection in mice; (2) intracerebral and intraperitoneal herpes simplex infection in mice; (3) intracerebral poliomyelitis (MEF_1) infection in mice; (4) intracerebral rabies infection in mice; and (5) intracoelomic and wing web Rous sarcoma infections in chicks. There was no difference between the infective titers of any of these viruses in treated and nontreated animals. There was no extension of survival time and thus treatment of the above 5 infections with ammonium picrate was ineffective.

To determine the *in vitro* effect of ammonium picrate, a 10^{-1} or 10^{-2} dilution of each stock virus was incubated for 1 hour at 37°C in the presence of concentrations of compound ranging in 10-fold steps from 10,000 μg to 1 μg/ml. The pH of the compound-virus mixtures ranged from pH 6.5-7.0. The infectivity of the incubated compound-virus mixtures was assayed by titration in animals and compared with that observed for control virus samples (without compound) incubated for the same period. There was no significant loss in infectivity in any of the control virus samples from incubation for 1 hour. The concentration of 10,000 mcg/ml inactivated influenza (PR8), herpes simplex, rabies and Rous sarcoma virus, but did not reduce the infectivity of poliomyelitis (MEF_1) virus. Lower concentrations of the compound had no effect on the infective titer of any of these

TABLE IV. Summary of *in vitro* and *in vivo* Antiviral Effect of Ammonium Picrate on 6 Viruses.

Virus	*In vitro* (mcg/ml)*	*In vivo* activity
Columbia SK	10	active
Influenza (PR8)	10,000	inactive
Herpes simplex	10,000	"
Poliomyelitis (MEF_1)	>10,000	"
Rabies	10,000	"
Rous sarcoma	10,000	"

* Minimum effective concentration at pH 6.5-7.0 and incubation for 1 hr at 37°C.

viruses. A 10^{-2} dilution of stock SK virus was completely inactivated at 10,000 μg/ml, a concentration of 10 μg/ml reduced the infectivity by at least 1000-fold and a concentration of 1 μg/ml was ineffective. Two attempts to recover viable SK virus from incubated compound-virus mixtures by the removal of ammonium picrate through dialysis resulted in failure. The results of the *in vitro* and *in vivo* tests with all 6 viruses are summarized in Table IV.

Discussion. The evidence obtained indicates that ammonium picrate specifically protects mice against the Columbia SK virus infection. This antiviral property was routinely demonstrable if two conditions were met: (1) treatment had to be given within 4 hours prior to, or at time of infection, and (2) the infecting virus dose had to be less than 100 LD_{95}. The action of the compound can probably be ascribed to a direct effect on the intact SK virus before it invades tissue cells. Findings in support of this assumption are: (1) the high degree of correlation between *in vitro* and *in vivo* activity; (2) the lack of activity when the compound was administered after the infection; (3) the lack of immunity in treated mice surviving the infection.

The reported experiments do not shed any light on how the compound and the SK virus interact after contact. Considering the relatively small amount of compound required to inactivate SK virus and the relatively large amounts required to inactivate 5 other viruses *in vitro,* one might suspect that a highly specific reaction is involved, rather than a nonspecific protein precipitation.

The specific activity of ammonium picrate *in vivo* can be attributed solely to the picrate ion, since picric acid as well as several other picrates were approximately equally effective, on a molar basis, against the SK virus infection.

Summary. Ammonium picrate has highly specific antiviral activity against a subcutaneously or intracerebrally induced, lethal Columbia SK virus infection in mice. The compound is active when given parenterally or orally; it also inactivates this virus *in vitro.* The compound has no effect on influenza type A (PR8), herpes simplex, poliomyelitis (MEF_1), rabies or Rous sarcoma virus infection in animals; it has only minimal effect against these viruses *in vitro.*

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